Environmental Health Perspectives
Vol. 23, pp. 329–331, 1978

Effect of Polybrominated Biphenyls on Adenylate Cyclase Activity in Rat Lung Alveoli

by Kirpal S. Sidhu* and Andrew M. Michelakis*

The *in vitro* effect of polybrominated biphenyls (FireMaster BP-6) on adenylate cyclase [ATP pyrophosphate lyase (cyclizing), EC 4.6.1.1] activity in plasma membranes of rat lung alveoli was determined. Two fractions of plasma membranes, PM-I (approximate d = 1.13 g/cm^3) and PM-II (approximate d = 1.16 g/cm^3) were isolated under hypotonic homogenization and fractionation conditions (1*mM* $NaHCO_3$, 5*mM* dithiothreitol, pH 7.5) by using differential centrifugation and nonlinear sucrose gradient centrifugation techniques. Polybrominated biphenyls (10 μg/ml) dissolved in ethyl acetate (0.25%) stimulated basal adenylate cyclase activity of plasma membranes by 2.1 to 2.5-fold. The implication of this observation is discussed.

Introduction

The fire retardant FireMaster BP-6 is a multicomponent commercial mixture of polybrominated biphenyls (PBB) produced by Michigan Chemical Corp. It contains 75% bromine by weight which is equivalent to hexabromobiphenyl. Electron-capture gas-chromatographic examination of this material indicates that it contains two major and at least six minor components (*1*). The two major components were identified by gas liquid chromatography and mass spectrometry as hexabromobiphenyl and an unresolved mixture of heptabromobiphenyl and octabromobiphenyl (*1*). The polybrominated biphenyls are insoluble in water but are soluble in fat solvents.

FireMaster BP-6 was accidently substituted for an approved animal feed additive in Michigan in 1973 (*2*). Several Michigan farmers observed a decrease in milk production of their herds in summer and fall of 1973 (*2*). The toxic syndrome associated with the accidental feeding of PBB to dairy cattle has been described by Jackson and Halbert (*3*). The main symptoms were loss of appetite, loss of body weight, abnormal hoof-nail growth, and mottled fur (*3*). The cause of this toxic syndrome could not be determined till early 1974 (*2, 4*). Since polybrominated biphenyls are fat-soluble and are excreted in fat parts of animal products (*5*), some farmers who consumed milk, meat, and eggs containing PBB were also contaminated (*2*). The effects of PBB contamination on human beings are not clear (*6*). Some farmers have reported a host of psychological, neurological, skin, and joint symptoms as PBB-related; however, no pattern of illness or symptoms has so far been correlated in a statistically significant manner with the concentration of PBB in blood (*6*).

The literature on metabolism of PBB in biological systems is very scanty. Babish et al. (*7*) have reported that several hepatic microsomal enzymes studied in Japanese quail are induced by dietary PBB.

Since many research workers have conclusively demonstrated that adenosine 3′,5′-cyclic monophosphate (cyclic AMP) acts as a central regulator at the molecular level of several diverse cell activities such as mitosis, metabolism (enzyme activation, transcription and translation), and responses to extracellular signals (*8*), we made efforts to study the effect of PBB on adenylate cyclase. Adenylate cyclase [ATP pyrophosphate lyase (cyclizing), EC 4.6.1.1] catalyzes the conversion of adenosine triphosphate (ATP) to cyclic AMP.

Reported herein is the *in vitro* effect of PBB on adenylate cyclase activity in plasma membranes of rat lung alveoli.

* Department of Pharmacology, College of Human Medicine, Michigan State University, East Lansing, Michigan 48824.

Materials and Methods

Materials

The [8-^{14}C] adenosine 5′-triphosphate tetrasodium salt (43.2 mCi/mmole) was purchased from International Chemical and Nuclear Pharmaceuticals, Inc., Irvine, California. Theophylline was obtained from Nutritional Biochemical, Cleveland, Ohio. Cyclic AMP, ATP, Tris, and dithiothreitol were purchased from Sigma Chemical Co., St. Louis, Missouri.

Isolation of Plasma Membranes

Lungs from Sprague-Dawley rats (150 to 200 g) that had been decapitated were excised and were allowed to remain in ice cold 0.85% NaCl. The lung tissue was dissected free from all visible bronchi and vasculature. The peripheral areas of lungs consisting predominantly of alveoli were isolated for preparation of plasma membranes. The isolation of plasma membranes from rat lung alveoli was adapted from the procedure of Neville (9). Two fractions of plasma membranes designated PM-I (approximate d = 1.13 g/cm^3) and PM-II (approximate d = 1.16 g/cm^3) were isolated under hypotonic conditions of homogenization and fractionation (1*mM* $NaHCO_3$, 5*mM* dithiothreitol, pH 7.5). The isolated plasma membrane fractions were suspended in an assay medium consisting of 0.02*M* glycylglycine, 0.01*M* $MgSO_4$, pH 7.4. The details of isolation and characterization of plasma membranes will be described elsewhere.

Adenylate Cyclase Assay

Adenylate cyclase was assayed by the method of Krishna et al. (*10*). Incubation was carried out at 30°C for 10 min in a Dubnoff metabolic shaker. The final concentration in reaction mixture (200 μl) was Tris (pH 7.4), 50.0*mM;* theophylline, 22.2*mM*; glycylglycine (pH 7.4), 7.5*mM*; bovine serum albumin 1.0 mg/ml; NaCl, 7.6*mM*; $MgSO_4$, 4.25*mM*; ATP containing 2 μCi [8-^{14}C] ATP, 1.25*mM;* and NaF, whenever included, 10.0*mM*. After termination of incubation, 62.5 nmole of cyclic AMP was added and the reaction was stopped by immersing the reaction tubes in the boiling water for 3 min. Cyclic AMP was purified by Dowex 50 [H^+] chromatography and $ZnSO_4-Ba(OH)_2$ precipitation (*10*). The ^{14}C radioactivity in an aliquot of supernatant added to 10 ml of scintillation mixture (*11*) was counted in a scintillation counter. The procedural losses were corrected by taking into consideration the recovery of cyclic AMP measured at 257 nm. The inclusion of theophylline, 22.2*mM*, in the reaction mixture of adenylate cyclase assay completely blocked the phosphodiesterase activity. Protein concentration was determined by the method of Lowry et al. (*12*).

The effect of fire retardant BP-6 on adenylate cyclase activity in plasma membranes of rat lung alveoli was determined. The final concentration of BP-6 in these assays was 10 μg/ml. During these studies, the effect of ethyl acetate on adenylate cyclase activity of plasma membranes was also evaluated. Ethyl acetate used as a diluent for BP-6 had a final concentration of 0.25% in the adenylate cyclase assay tubes.

Results and Discussion

The *in vitro* effect of polybrominated biphenyls (FireMaster BP-6) on adenylate cyclase activity in plasma membranes of rat lung alveoli is shown in Table 1. Basal adenylate cyclase activity of plasma membranes was stimulated by NaF (0.01*M*). The stimulation of adenylate cyclase activity by NaF

Table 1. *In vitro* effect of polybrominated biphenyls (FireMaster BP-6) on adenylate cyclase activity in plasma membranes of rat lung alveoli.

	Adenylate cyclase activity in plasma membrane fractions[a]			
	PM-I		PM-II	
	pmole CAMP/min mg protein	Stimulation ratio	pmole CAMP/min mg protein	Stimulation ratio
NaCl, 0.85% (Basal)	194.1	1.00	148.0	1.00
NaF, 0.01*M*	742.0	3.82	576.2	3.89
BP-6, 10 μg/ml + ethyl acetate, 0.25%	408.5	2.10	373.3	2.52
Ethyl acetate, 0.25%	255.2	1.31	141.6	0.96

[a] The final concentration in the reaction mixture (200 μl) for adenylate cyclase was Tris (pH 7.4), 50*mM;* theophylline, 22.2*mM;* glycylglycine (pH 7.4), 7.5*mM;* bovine serum albumin, 1.0 mg/ml; NaCl, 7.6*mM;* $MgSO_4$, 4.25*mM;* and ATP containing 2 μCi [8-^{14}C] ATP, 1.25*mM*. Each value represents mean of two experiments.

has been consistently observed in various mammalian tissues (*13*). Polybrominated biphenyls (10 μg/ml) also appeared to stimulate the basal adenylate cyclase activity of plasma membranes of rat lung alveoli. However, we wish to emphasize that these results represent a preliminary finding on the basis of two *in vitro* experiments. The application of this *in vitro* observation to the conditions *in vivo* remains to be studied.

The implications of this observation are not clear. In general, the effect of high intracellular cyclic AMP concentration is to reduce immunologic reactivity with inhibitory effects on cellular proliferation, chemotaxis, mediator release, and end organ responsiveness (*14*). However, in lymphocyte transformation, B cell proliferation and chemotaxis there is also evidence for a stimulatory action of cyclic AMP (*15*).

An increase in ratio of guanosine 3',5'-cyclic monophosphate (cyclic GMP) to cyclic AMP results in an increase in cell growth and division, whereas a decrease in the ratio results in decrease of these functions (*16*). In view of this discussion, it seems desirable to study the effect of PBB on the ratio of cyclic GMP to cyclic AMP. The effects of PBB on immunologic processes should also be studied.

The authors wish to thank Mr. Daniel J. Murfin for his excellent technical assistance and to Ms. Sharon Hallabrin for her help in the preparation of the manuscript. We are grateful to Dr. Harold Price for his gift of polybrominated biphenyls (FireMaster BP-6).

REFERENCES

1. Fries, G. F., and Marrow, G. S. Excretion of polybrominated biphenyls into the milk of cows. J. Dairy Sci. 58: 947 (1975).
2. Carter, L. J. Michigan's PBB incident: Chemical mix-up leads to disaster. Science 192: 240 (1976).
3. Jackson, T. F., and Halbert, F. L. A toxic syndrome associated with the feeding of polybrominated biphenyl-contaminated protein concentrate to dairy cattle. J. Amer. Vet. Med. Assoc. 165: 437 (1974).
4. Fehringer, N. V. Determination of polybrominated biphenyl residues in dairy products. J. Assoc. Offic. Agr. Chem. 58: 978 (1975).
5. Norris, J. M., et al. Toxicological and environmental factors involved in the section of decabromodiphenyl oxide as a fire retardant chemical. J. Fire Flammability/Combustion Toxicol. 1: 52 (1974).
6. Anonymous. Michigan screens blood for PBB contamination. J. Environ. Health 39: 436 (1977).
7. Babish, J. G., Gutenmann, W. H., and Stoewsand, G. S. Polybrominated biphenyls: Tissue distribution and effect on hepatic microsomal enzymes in Japanese quail. J. Agr. Food Chem. 23: 879 (1975).
8. Robison, A. G., Butcher, R. W., and Sutherland, E. W. Cyclic AMP. Academic Press, New York, 1971.
9. Neville, D. M., Jr. The isolation of cell membrane fraction from rat liver. J. Biophys. Biochem. Cytol. 8: 413 (1960).
10. Krishna, G., Weiss, B., and Brodie, B. B. A simple sensitive method for the assay of adenyl cyclase. J. Pharmacol. Exptl. Therap. 163: 379 (1968).
11. Bray, G. A. A simple efficient liquid scintillator for counting aqueous solutions in a liquid scintillation counter. Anal. Biochem. 1: 279 (1960).
12. Lowry, O. H., et al. Protein measurement with the folin phenol reagent. J. Biol. Chem. 193: 265 (1951).
13. Perkins, J. P. Adenyl cyclase. Adv. Cyclic Nucleotide Res. 3: 1 (1973).
14. Parker, C. W. Cyclic AMP. In: Inflammation: Mechanism and Control. I. H. Lepow and P. A. Ward, Eds., Academic Press, New York, 1972.
15. Parker, C. W., Sullivan, T. J., and Wedner, H. J. Cyclic AMP and the immune response. Adv. Cyclic Nucleotide Res. 4: 1 (1974).
16. Latner, A. L. In: Cantarow and Trumper Clinical Biochemistry, Saunders, Philadelphia, 1975, p. 251.